Attorney Docket No. 02481.1671-01



Application No.: 09/991,964

- 25. The process as claimed in claim 23, wherein the protein is a heterologous protein prepared in a microorganism.
- 26. The process as claimed in claim 25, wherein the microorganism is a bacterium.
- 27. The process as claimed in 26, wherein the bacterium is Escherichia coli.
- 28. The process as claimed in claim 25, wherein the microorganism is a yeast.
- 29. The process as claimed in claim 28, wherein the yeast is *Saccharomyces* cerevisiae.
- 30. The process as claimed in claim 28, wherein the yeast is *Pichia pastoris*.
- 31. The process as claimed in claim 23, wherein the protein is a heterologous protein and is prepared in an insect cell.
- 32. The process as claimed in claim 25 or claim 31, wherein the protein is prepared from an expression vector construct.
- 33. The process as claimed in claim 23, wherein the protein is present in dissolved form.
- 34. The process as claimed in claim 23, wherein the protein is present in suspension.



FINNEGAN HENDERSON FARABOW GARRETT& DUNNER LLP

Attorney Docket No. 02481.1671-01

Application No.: 09/991,964

35. The process as claimed in claim 23, wherein cysteine is added in a concentration of from about 100 mM to the aqueous solution.

36. The process as claimed in claim 23, wherein cysteine is added in a concentration

of greater than 100 mM to the aqueous solution.

37. The process as claimed in claim 23, wherein the concentration of cysteine in the

aqueous protein solution is in the range from about 100 mM to about 500 mM.

38. The process as claimed in claim 37, wherein the concentration of cysteine in the

aqueous protein solution is in the range from about 150 mM to about 220 mM.

39. The process as claimed in claim 37, wherein the concentration of cysteine in the

aqueous protein solution is about 170 mM.

40. The process as claimed in claim 23, wherein the storage of the protein takes

place at about 0°C to about 50°C.

41. The process as claimed in claim 40, wherein the storage of the protein takes

place at about 5°C to about 30°C.

42. The process as claimed in claim 41, wherein the storage of the protein takes

place at about 5°C.

FINNEGAN HENDERSON FARABOW GARRETT & DUNNER LLP

- **Application No.:** 09/991,964
- 43. The process as claimed in claim 23, wherein the protein stored is insulin, an insulin derivative, or a precursor thereof.
- 44. A process for the preparation and storage of a heterologous protein, comprising the expression of the heterologous protein or its precursor in a transformed microorganism, optional disruption of the microorganism and/or isolation of the heterologous protein or its precursor from the culture medium, and the subsequent storage of the heterologous protein according to the process of claim 23.
- 45. The process of claim 44, further comprising the renaturation of the heterologous protein or its precursor and the purification and isolation of the heterologous protein, including optional removal of a leader sequence or other sequences that may be present in the precursor of the heterologous protein.
- 46. The process as claimed in claim 44, wherein the heterologous protein is animal insulin.
- 47. The process as claimed in claim 45, wherein the animal insulin is human insulin.
- A process for the storage of a protein in an aqueous solution, comprising adding an amount of cysteine effective to delay the temporal decrease in the effective concentration of the protein during a period of greater than 24 hours.

Bl

FINNEGAN HENDERSON FARABOW GARRETT& DUNNER LLP

- **Application No.:** 09/991,964
- 49. The process as claimed in claim 48, wherein the temporal decrease in the effective concentration of the protein is delayed for a period of 48 hours or more.
- 50. The process as claimed in claim 48, wherein the temporal decrease in the effective concentration of the protein is delayed for a period of 1 week or more.
- 51. The process as claimed in claim 48, wherein the temporal decrease in the effective concentration of the protein is delayed for a period of 2 weeks or more.
- 52. The process as claimed in claim 48, wherein the temporal decrease in the effective concentration of the protein is delayed for a period of 4 weeks or more.
- 53. The process as claimed in claim 48, wherein the temporal decrease in the effective concentration of the protein is delayed for a period of 8 weeks or more.
- 54. The process as claimed in claim 48, wherein the temporal decrease in the effective concentration of the protein is delayed for a period of from greater than 24 hours to 2 months.
- 55. The process as claimed in claim 48, wherein the protein is a heterologous protein prepared in a microorganism.
- 56. The process as claimed in claim 55, wherein the microorganism is a bacterium.
- 57. The process as claimed in claim 56, wherein the bacterium is Escherichia coli.



FINNEGAN HENDERSON FARABOW GARRETT& DUNNER LLP

Attorney Docket No. 02481.1671-01

Application No.: 09/991,964

58. The process as claimed in claim 55, wherein the microorganism is a yeast.

59. The process as claimed in claim 58, wherein the yeast is *Saccharomyces* cerevisiae.

60. The process as claimed in claim 58, wherein the yeast is *Pichia pastoris*.

61. The process as claimed in claim 48, wherein the protein is a heterologous protein and is prepared in an insect cell.

62. The process as claimed in claim 55 or claim 61, wherein the protein is prepared from an expression vector construct.

63. The process as claimed in claim 48, wherein the protein is present in dissolved form.

64. The process as claimed in claim 48, wherein the protein is present in suspension.

65. The process as claimed in claim 48, wherein cysteine is added in a concentration of from about 100 mM to the aqueous solution.

66. The process as claimed in claim 48, wherein cysteine is added in a concentration of greater than 100 mM to the aqueous solution.

Cont

FINNEGAN HENDERSON FARABOW GARRETT& DUNNER LLP

- 67. The process as claimed in claim 48, wherein the concentration of cysteine in the agueous protein solution is in the range from about 100 mM to about 500 mM.
- 68. The process as claimed in claim 67, wherein the concentration of cysteine in the aqueous protein solution is in the range from about 150 mM to about 220 mM.
- 69. The process as claimed in claim 67, wherein the concentration of cysteine in the aqueous protein solution is about 170 mM.
- 70. The process as claimed in claim 48, wherein the storage of the protein takes place at about 0°C to about 50°C.
- 71. The process as claimed in claim 70, wherein the storage of the protein takes place at about 5°C to about 30°C.
- 72. The process as claimed in claim 70, wherein the storage of the protein takes place at about 5°C.
- 73. The process as claimed in claim 48, wherein the protein stored is insulin, an insulin derivative, or a precursor thereof.
- 74. A process for the preparation and storage of a heterologous protein, comprising the expression of the heterologous protein or its precursor in a transformed microorganism, optional disruption of the microorganism and/or isolation of the

Bl

FINNEGAN HENDERSON FARABOW GARRETT& DUNNER LLP

heterologous protein or its precursor from the culture medium, and the subsequent storage of the heterologous protein according to the process of claim 48.

Application No.: 09/991,964

- 75. The process of claim 74, further comprising the renaturation of the heterologous protein or its precursor and the purification and isolation of the heterologous protein, including optional removal of a leader sequence or other sequences that may be present in the precursor of the heterologous protein.
- 76. The process as claimed in claim 75, wherein the heterologous protein is animal insulin.

The process as claimed in claim 75, wherein the animal insulin is human insulin.

REMARKS

Claims 2-22 were cancelled upon filing this application. By this preliminary amendment, claim 1 is cancelled and new claims 23-77 are added. These amendments do not introduce new matter.

Support for claims 23-47 may be found in the specification as a whole, and especially at pages 9-10, tables 1-4.

In particular, in table 3, the effective concentration of the protein in mg/L after 8 weeks of storage is about 93% of that of the first day. In table 1, the amount of valuable protein after 8 weeks of storage is about 97% of that of the first day.

FINNEGAN HENDERSON FARABOW GARRETT& DUNNER LLP